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FURTHER STUDIES OF AN ANTIPOLIOMYELITIC SERUM, ITS PROTECTIVE AND CURATIVE PROPERTIES IN EXPERIMENTAL POLIOMYELITIS OF MONKEYS

John W. Nuzum and Ralph G. Willy

From the Pathologic Laboratory of the Cook County Hospital, Chicago

Extended observations over a considerable number of years have established the fact that one attack of poliomyelitis confers immunity against subsequent infection. Levaditi and Landsteiner[1] and Flexner and Lewis[2] have shown that the serum of recovered patients and monkeys may have acquired neutralizing and protective properties against the virus of poliomyelitis. The application of this knowledge to the treatment of poliomyelitis in man was made first by Netter[3] and more recently by others, most of whom appear to be favorably impressed with the results. Unfortunately there are several disadvantages attendant on the method of treating poliomyelitis with the serum of recovered persons. Chief among these is the difficulty in securing serum in sufficient quantities to properly treat any considerable number of cases, and the probable low antibody content of human serum.

An intensive bacteriologic study of some 250 cases of acute epidemic poliomyelitis in Chicago during the summer of 1917 has served to confirm with striking regularity the presence of the same peculiar coccus which was isolated quite constantly the previous summer both from the brain and spinal cord,[4] and also from the cerebrospinal fluid[5] in human poliomyelitis.

It was first shown by Nuzum[6] that repeated injections of the poliomyelitic coccus intravenously in animals produce antibodies in high titer, chiefly agglutinins, opsonins and complement fixation bodies. Furthermore, in a small series of experiments with monkeys, young

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[1] Compt. rend. Acad. d. sc., 1910, 150, p. 131.

[2] Jour. Am. Med. Assn., 1910, 54, p. 1780.

[3] Arch. de méd. d. enfants, 1916, 19, p. 1.

[4] Mathers, George: Jour. Infect. Dis., 1917, 20, p. 113. Nuzum, J. W., and Herzog, Maximilian: Jour. Am. Med. Assn., 1916, 67, p. 1205. Rosenow, E. C., Towne, E. B., and Wheeler, G. W.: Jour. Am. Med. Assn., 1916, 67, p. 1202.

[5] Nuzum, J. W.: Jour. Am. Med. Assn., 1916, 67, p. 1437.

[6] Jour. Am. Med. Assn., 1917, 68, p. 24.

rabbits and white mice, injections of immune sheep serum appeared to confer a limited degree of protection against fatal doses of recently isolated virulent poliomyelitic cocci. Still more recently Nuzum and Willy[7] have reported very favorable results in a series of 159 cases of poliomyelitis treated with antipoliomyelitic horse serum in the Cook County Hospital during the summer of 1917.

It now appears advisable to determine the question whether or not such a serum prepared by immunizing animals with the coccus of poliomyelitis has protective or curative properties for monkeys inoculated with fatal doses of highly virulent virus. Rosenow[8] has recently reported the production of an antipoliomyelitic serum in horses immunized with poliomyelitic monkey coccus, which appears to have neutralizing, protective and curative properties against the virus.

A detailed study of the protective and curative properties of a serum produced by injection of poliomyelitic serum in experimental poliomyelitis in monkeys forms the basis of this paper.

IMMUNIZATION OF HORSE

A large horse, 6 years old, weighing 1,400 lbs., was selected and immunized, at first, according to the method described by Amoss and Wollstein,[9] which method we previously employed in the immunization of sheep. The horse received intravenous injections of living organisms in increasing doses on 3 successive days, followed by periods of rest of 7-10 days. For the first injection the 24-hour growth on a dextrose agar slant was suspended in 2 c c of sterile salt solution and 0.3 c c of this suspension in 10 c c of salt solution was injected intravenously. Twenty-four hours later 0.5 c c of the foregoing suspension of bacteria in 10 c c salt solution was injected intravenously and a similar dose given on the 3rd day. This was followed by a period of rest when a second series of injections were instituted. Small desensitizing doses were given during the first injection of each series and after the lapse of an hour the full dose was given. The organisms injected have all been originally cultured in 1% dextrose broth and later on dextrose agar slants and incubated at 35 C. for 18-24 hours. All of the strains employed for immunization have been subcultured through several generations, always under aerobic conditions, and each strain injected has been plated regularly on blood agar prior to injection into the horse.

The first serial injections were begun Jan. 30, 1917, and consisted of the poliomyelitic coccus isolated from the brain and cords of two recent human cases. The following 6 serial injections contained strains isolated from the spinal fluid and the central nervous system of 6 fatal cases of poliomyelitis, all of which were confirmed by microscopic sections of the spinal cord. The next 7 serial injections consisted chiefly of the coccus isolated from the central nervous system of 5 different monkeys recently paralyzed with virus. The injections were given usually intravenously, but also intramuscularly and subcutaneously into the neck, shoulders and buttocks. The maximum dose consisted of the 24-hour surface growth of 3 dextrose agar slants in 8 ounce bottles. Regular serial injections of both human and monkey coccus were continued up to July 1, 1917. A test bleeding, June 6, revealed a potent immune serum. At this time 5,000 units of tetanus antitoxin were injected subcutaneously. The horse has been bled as follows: June 6, 500 c c; July 11, 2,000 c c; July 28, 2,000 c c; Aug. 8, 3,500 c c; Aug. 23, 2,500 c c.

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[7] Jour. Am. Med. Assn., 1917, 69, p. 1247.

[8] Ibid., p. 261.

[9] Jour. Exper. Med., 1916, 23, p. 403.

PROTECTION OF MONKEYS AGAINST POLIOMYELITIS WITH IMMUNE HORSE SERUM

It is well known that by injecting suitable doses of virulent monkey-adapted virus, the percentage of takes following intracerebral injections is 100%. In the experiments recorded in the accompanying tables we have used a moderately virulent monkey-adapted virus which was passed through 12 successive generations of monkeys in the Hygienic Laboratory, Washington, D. C., and kindly sent us by Dr. N. E. Wayson, of the Public Health Service, Washington, D. C. We passed this virus through 4 additional monkeys and used it in all of the experiments as Virus 25 (15 Generation) and Virus 27 (16 Generation). It has been injected in doses of 1 c c of a 5% emulsion which represents at least 2 fatal doses for all monkeys not immune.

In the experiments in Table 1 all of the monkeys except 53 (0.5 c c virus) received an intracerebral injection of 1 c c of the same 5% emulsion of recently glycerolated Virus 25 under ether anesthesia. Simultaneous injections of 10-20 c c immune serum intravenously 2 to 3 c c intraspinally and occasionally intramuscular injections of 10 to 15 c c of immune horse serum, were given for 2 successive days, followed by a lapse of a day, and then for 2 additional days. Monkeys 49 and 50 each received a total of 82 c c immune serum and were completely protected not only against the first injection but also against subsequent injections of virus. Monkeys 44 to 47, inclusive, each received immune serum in amounts varying from 80-110 c c. Monkeys 46 and 47 resisted the first inoculation but became severely paralyzed following the second injection of virus. Monkey 48 has no value in this series of experiments since the necropsy revealed that death was due to a traumatic intracerebral hemorrhage the 2nd day following inoculation. Monkey 52 (control) received injections of a total of 100 c c of pooled normal horse and sheep serum. He became severely paralyzed and died with typical microscopic lesions of poliomyelitis. Monkey 53 (control) inoculated with 0.5 c c of virus, developed paralysis of all extremities and died on the 4th day. It thus appears that 6 monkeys treated with immune serum were completely protected.

TABLE 1

Protection of Monkeys Against Poliomyelitis with Immune Horse Serum
(Neutralization of Virus "In Vivo")

Monkey	Intracerebral Inoculation	Treatment	Result
44	1 c c Virus 25	Simultaneous intraspinal, intramuscular and intravenous injections of immune horse serum	No symptoms for 31 days. Has remained well following second inoculation
45	1 c c Virus 25	Simultaneous intraspinal, intramuscular and intravenous injections of immune horse serum	No symptoms for 21 days. Resisted a second inoculation
46	1 c c Virus 25	Simultaneous intraspinal, intramuscular and intravenous injections of immune horse serum	No symptoms for 21 days; severe paralysis 12th day following second inoculation. Recovery following injections of immune horse serum
47	1 c c Virus 25	Simultaneous intraspinal, intramuscular and intravenous injections of immune horse serum	No symptoms first inoculation; severely paralyzed 4th day following second inoculation. No improvement following injections of immune horse serum. Died 7th day
48	1 c c Virus 25	Control Injections of pooled normal horse serum	Found dead in cage 2nd day following inoculation. Necropsy revealed traumatic intracerebral hemorrhage at site of inoculation. No microscopic evidence of poliomyelitis
49	1 c c Virus 25	Simultaneous intraspinal and intravenous injections of immune horse serum	No symptoms following first inoculation. Has remained well following second and third inoculations
50	1 c c Virus 25	Simultaneous intraspinal and intravenous injections of immune horse serum	No symptoms following first inoculation. Has remained well following second and third inoculations
51	1 c c Virus 25	Control Repeated intraspinal injections of normal sheep serum	Tremor and hyperirritability 3rd day after inoculation followed by severe paralysis and death. Microscopically, typical poliomyelitis
52	1 c c Virus 25	Control Repeated intravenous and intraspinal injections of pooled normal horse and sheep serum	Symptoms 9th day; severe paralysis 10th day rapidly followed by typical respiratory paralysis and death same day. Microscopically, marked typical lesions of poliomyelitis
53	0.5 c c Virus 25	Control None	Symptoms 3rd day after inoculation rapidly followed by paralysis and death 4th day. Microscopically, typical poliomyelitis

CURATIVE PROPERTIES OF IMMUNE SERUM AFTER THE ONSET OF SYMPTOMS OF POLIOMYELITIS

The experiments summarized in Table 2 reveal that 7 of the series of 10 monkeys treated with immune serum after the onset of poliomyelitis recovered; that is, a recovery rate of 70%. It is well known that poliomyelitis in monkeys has a death rate of from 76-100%. Five

TABLE 2

CURATIVE PROPERTIES OF IMMUNE SERUM AFTER ONSET OF SYMPTOMS OF POLIOMYELITIS

Monkey	Inoculation	Symptoms at Beginning of Treatment	Treatment and Total Amount of Serum Injected	Clinical Picture after Serum	Result
25	1 c c Virus 20 intraspinally	Tremor, ptosis, flaccid paralysis of both legs 7th day after inoculation. Eighth day monkey completely paralyzed in all extremities. Can only lift up head	44 c c immune serum injected intravenously, intraspinally and intramuscularly over period of next 5 days	Twelfth day: Tremor gone; monkey appears bright, takes food and drink eagerly. Completely paralyzed and can only move head	Survived
27	1 c c Virus 25 intracerebrally	Ninth day: Fine muscular tremor; flaccid paralysis of hind legs; paresis of right arm with wrist drop	60 c c immune horse serum injected as above	Tenth day: Intercostal muscles paretic; respirations rapid and labored; monkey cyanotic	Died
28	1 c c Virus 25 cerebrally	Tenth day: Flaccid paralysis of both legs; tremor and weakness of arms	80 c c immune horse serum injected as above	Thirteenth day: Tremor and fever gone; eats well; permanent paralysis of all extremities	Lived
33	1 c c Virus 27	Sixth day: General muscular weakness and stupor. Seventh day: Severely paralyzed in both legs and arms	55 c c immune horse serum injected as above	Tenth day: Appears bright; stupor gone; eats ravenously; severe residual paralysis	Lived
34	1 c c Virus 27	Sixth day: Excitable; fine muscular tremor; weak in legs	75 c c immune serum as above	Marked residual paralysis; lived 13 days after symptoms began	Favorable
54	1 c c Virus 27	Third day: Preparalytic symptoms; monkey is tremulous and very excitable. Spinal fluid clear; 330 cells per c.mm., chiefly lymphocytes. Noguchi test strongly positive	10 c c serum intravenously 3 c c immune serum intraspinally	Fifth day: Appears normal; has remained free from paralysis to date	Lived
57	1 c c Virus 27	Fourth day: Preparalytic symptoms; irritable; climbs slowly and hind legs appear weak. Spinal fluid clear; 524 cells per c.mm., chiefly lymphocytes. Positive Nonne and Noguchi	10 c c serum intravenously on 4th day. Tenth day: 22 c c of immune serum	Fifth day: Improved. Tenth day: Return of symptoms; tremor and flaccid paralysis of both legs	Lived
55	1 c c Virus 27	Third day: Preparalytic symptoms; monkey highly exciting; fine muscular tremor. Spinal fluid clear; 220 cells per c.mm., 80% lymphocytes, 20% polynuclears. Positive globulin test	12 c c serum intravenously; 2 c c serum intraspinally	Recovered; has remained free from paralysis	Recovery
56	1 c c Virus 27	Fourth day: Preparalytic symptoms; irritable and excitable. Spinal fluid opaque; 618 cells per c.mm., 80% lymphocytes. Strong Noguchi test	20 c c serum intravenously; 3 c c spinally	Has remained well to date	Recovery
58	1 c c Virus 27	Fourth day: Monkey prostrate in cage; very tremulous; flaccid paralysis of both legs	40 c c immune serum by intravenous injections	Transient improvement. Stupor and death 3rd day	Died

monkeys inoculated with virus in the usual manner received no treatment and all died within periods varying from 4 hours to 5 days after the onset of paralysis.

It will be noted that Monkeys 54 to 57, inclusive, were treated in the preparalytic stage before the onset of a flaccid paralysis but only after unmistakable symptoms and definite changes in the cerebrospinal fluid. One of this group, Monkey 57, was treated in the preparalytic stage with a single intravenous injection of 10 c c immune serum. The animal appeared to recover and remained apparently entirely normal for a period of 5 days. However, on the 10th day after the inoculation of virus the monkey developed a severe paralysis, indicating that a single injection of serum was inadequate. Recovery followed an additional injection of serum.

From Table 2 it is seen that a single dose of immune horse serum sufficed in three instances to prevent paralysis when administered in the preparalytic stage, whereas repeated doses frequently were required to save the animal's life after the onset of paralysis. Furthermore, in all of this series of monkeys except Monkey 27, injections of immune serum regularly effected a striking improvement in the clinical picture of the disease within 24-48 hours after administration. The stupor disappeared, and the fever and the muscular tremor subsided. Usually at the end of 48 hours the monkey took food and drink eagerly, although he was frequently so severely paralyzed as to be unable to lift his head from the floor of the cage. The degree of actual paralysis present at the time of treatment has always been permanent, in our experience.

IMMUNIZATION OF MONKEYS BY REPEATED INJECTIONS OF IMMUNE SERUM PRIOR TO INOCULATION WITH VIRUS

Table 3 includes a series of 5 monkeys immunized over periods varying from 10-28 days as indicated. Monkeys 33, 34 and 37 received repeated injections of immune horse serum. Monkey 39 (control) was treated with a total of 125 c c of pooled normal horse serum. Monkey 28 (control) received a total of 90 c c of mixed normal horse and normal sheep serum over a period of 16 days prior to inoculation with virus. July 19, all of these monkeys were inoculated intracerebrally, under ether anesthesia, with 1 c c of the same 5% emulsion of glycerolated brain and cord of Virus 25. Monkeys 33, 34 and 37 proved to be immune to the first inoculation, whereas the control treated with normal serum became severely paralyzed. Further proof that infection did not occur following the first inoculation of Monkeys 33 and 34 is afforded by the fact that each of these animals became severely paralyzed following a second inoculation with virus. Monkey 37 resisted 2 different inoculations of virus but developed a severe paralysis following the third inoculation. This monkey was protected against a fatal issue by injections of a mixture of the serum of 2 paralyzed monkeys whose blood was known to possess a neutralizing effect on virus.

From Table 3 it thus appears that 3 monkeys receiving repeated injections of immune serum prior to inoculation with virus were completely protected against one inoculation of virus while the controls similarly treated but with normal serum became severely paralyzed.

TABLE 3

Immunization of Monkeys Against Virulent Virus by Repeated Injections of Antipoliomyelitic Serum

Monkey	Amount of Serum	Inoculation	Result
33 M. rhesus	June 21 to July 19: 225 c c immune horse serum injected intravenously, intramuscularly and intraspinally	July 19: 1 c c 5% emulsion of Virus 25 intracerebrally Aug. 11: Second inoculation 1 c c 5% emulsion of Virus 27 intracerebrally	No symptoms. Well 33 days after first inoculation Aug. 17, 8 a. m.: Monkey prostrate in cage; flaccid paralysis of all extremities; marked tremor. Protection after 60 c c immune serum
34 M. rhesus	July 9 to July 19: 140 c c immune horse serum by intravenous, intramuscular and intraspinal injections	July 19, noon: 1 c c 5% emulsion of Virus 25 Aug 11, noon: 1 c c 5% emulsion of Virus 27	No symptoms 23 days after first inoculation Aug. 17, 1 p. m.: Paralyzed in hind legs; weakness of right arm; improvement following 75 c c immune horse serum
37 Ring Tail	July 13 to Aug. 2: 123 c c immune serum injected as above	Ang. 2: 1 c c 5% emulsion of Virus 25 Aug. 11, a. m.: 1 c c 5% emulsion of Virus 27 Aug. 22, noon: 1 c c 5% emulsion of Virus 27	No symptoms Remained well Aug. 25: Tremor and excitability followed by flaccid paralysis of both legs. Aug. 27: Protection following intravenous and intraspinal injections of immune Monkey Serums 16 and 18
Control 39 Ring Tail	July 10 to July 19: 125 c c pooled normal horse serum injected intravenously, intraspinally and intramuscularly	July 19, 1:30 p. m.: 1 c c 5% emulsion of Virus 25 intracerebrally	July 22: Onset of symptoms rapidly followed by paralysis and death July 24. Microscopically, typical lesions of acute poliomyelitis
Control M. rhesus 28	90 c c of mixed normal horse and normal sheep serum injected intravenously, intramuscularly over period of 16 days	1 c c Virus 25 5% emulsion intracerebrally	Symptoms 9th day after inoculation; 10th day severely paralyzed in all extremities; stuporous. Recovery followed injection of 80 c c immune horse serum. Living 51 days later with marked atrophy of all extremities

THE OPSONIC CONTENT OF IMMUNE HORSE SERUM AND OF SERUM OF PARALYUED MONKEYS

Over 80 estimations have been made of the opsonic content of immune horse serum and of the serum of paralyzed monkeys for different strains of the poliomyelitic coccus isolated both from human and monkey sources. In this work we have used the dilution method to determine at what point induced phagocytosis definitely exceeded spontaneous phagocytosis. The washed leukocytes of the same normal adult person have been used in all of these determinations. The degree of spontaneous phagocytosis was estimated by the amount of phagocytosis in specimens of salt solution instead of serum. In these experiments we used typical strains of poliomyelitic cocci isolated from the spinal fluid of patients both before and after death, from the central nervous system of fatal human cases and also from the brain and cord of paralyzed monkeys.

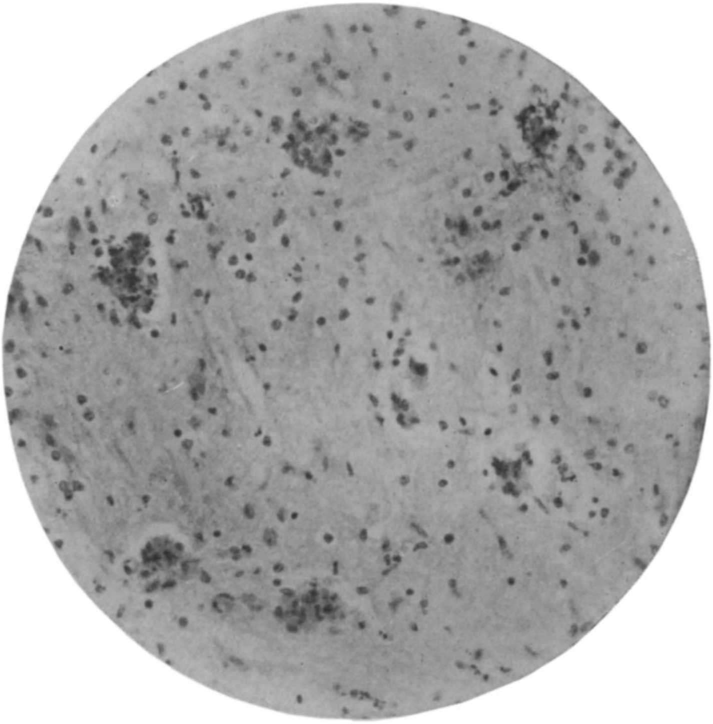

Fig. 1.—Illustrating typical neurophagocytosis in the ganglion cells of the lumbar level of the spinal cord of Monkey 27. This monkey was a control inoculated with virus in the usual manner and treated with a total of 90 c c of pooled normal horse and sheep serum. × 230.

As controls and to determine the specificity of the reaction, we used 2 strains of Streptococcus viridans from cases of acute endocarditis, a hemolytic streptococcus, 10 strains of streptococci from scarlet fever, 1 strain of pneumococci and 2 strains of Staphylococcus aureus and Staphylococcus albus. Suspensions of the organisms were prepared by washing off the 24-hour surface growth of an agar slant and diluting with saline solution to approximately the same turbidity. After incubation of the tubes at 35 C. for 30 minutes, 50 polynuclear leukocytes were counted and the number of cells engaged in phagocytosis determined.

The point of opsonic extinction of the immune horse serum as estimated with homologous strains of poliomyelitic cocci has reached 1:6,000 both for human and monkey strains of cocci. Moreover, by means of immune horse serum we have been able in 20 instances to identify and separate strains of poliomyelitic cocci recently isolated from the spinal fluid of man from other strains of cocci presenting more or less confusing cultural and morphologic similarities. Furthermore, the serum of 6 paralyzed monkeys recovering after treatment with immune horse serum have all shown a high specific opsonic content both for human and monkey strains of the poliomyelitic coccus.

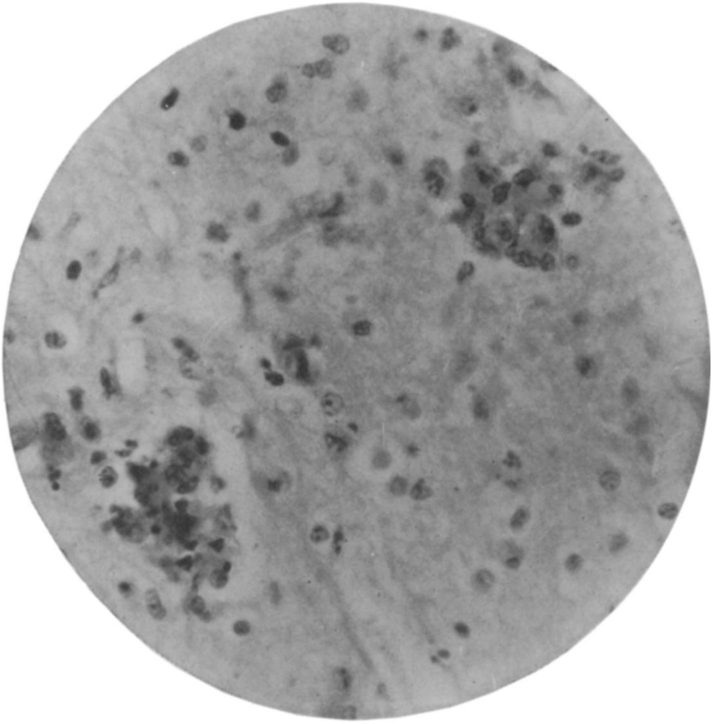

Fig. 2.—Same as Fig. 1. × 500.

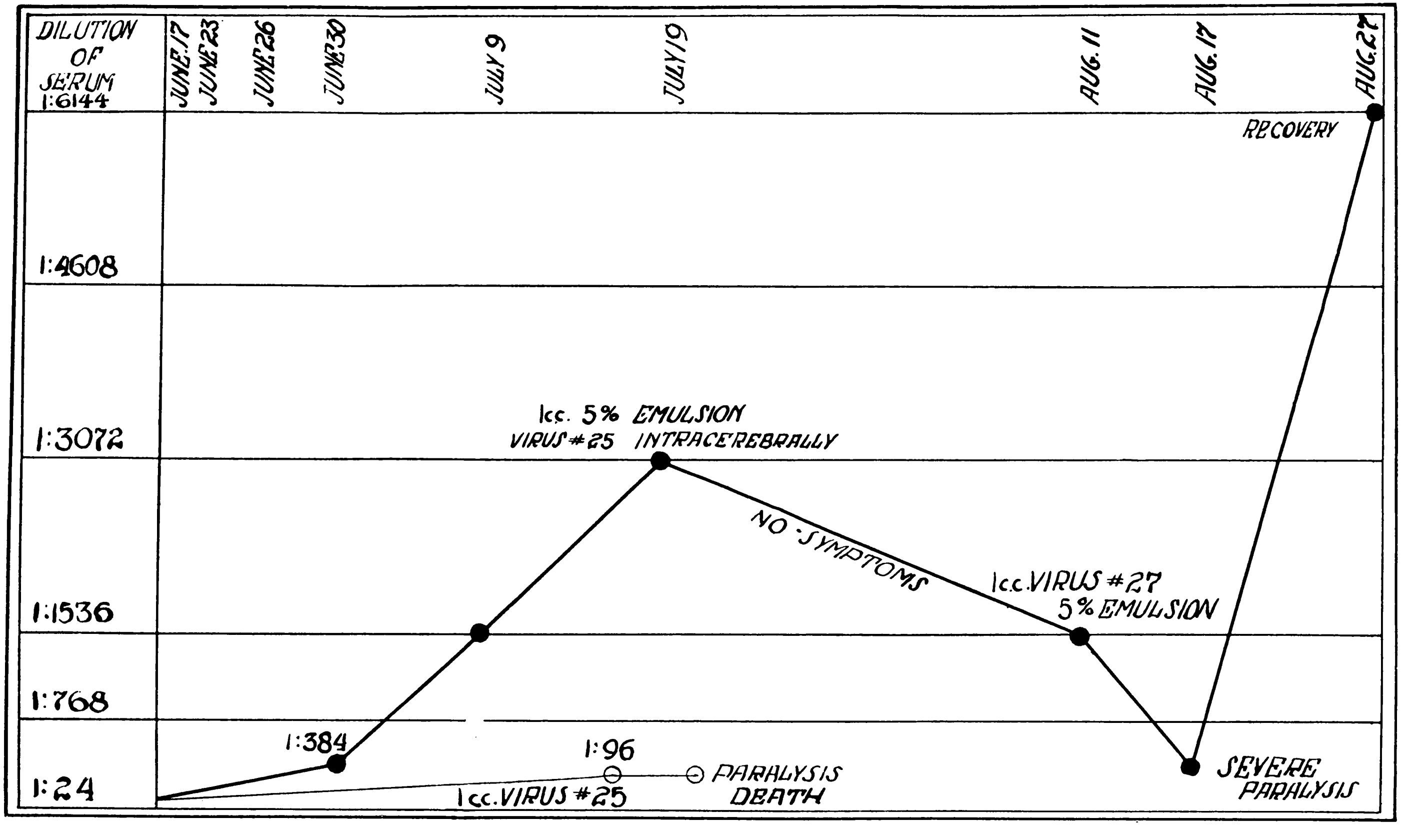

Chart 1.—The rise in the opsonin content of the serum of a monkey receiving injections of immune horse serum. Note the corresponding fall in the opsonic titer following injections of virulent virus.

Mathers and Tunnicliff[10] have shown a specific increase in the opsonin content of the serum of convalescent children for the coccus of poliomyelitis.

The heavy line on Chart 1 illustrates the rise in the opsonin content of the serum of Monkey 33, (M. rhesus) following repeated injections of immune horse serum. The light line represents the point of opsonic extinction for the poliomyelitis coccus in the serum of a control monkey treated with injections of pooled normal horse serum. Injections of immune serum were given intravenously, intramuscularly and intraspinally as follows: June 17, 50 c c serum; June 23, 25 c c; June 26, 44 c c ; June 30, 35 c c; July 10, 75 c c immune horse serum. July 19, the opsonin titer of the serum of Monkey 33 was 1:3072, while the point of opsonic extinction for the poliomyelitic coccus in the serum of the control monkey was but slightly above normal, that is, 1:96. At this time each monkey received an injection of Virus 25 intracerebrally. The control monkey became severely paralyzed and died the 4th day after inoculation. Monkey 33 was immune and after a lapse of 3 weeks the opsonin content of his serum had fallen to 1:1,536. At this time, Aug. 11, he received a second inoculation of Virus 27 and became severely paralyzed in all extremities on the 6th day. This monkey recovered following injections of 60 c c immune horse serum. Ten days after the onset of the paralysis and at a time when the animal had been actively immunized against subsequent injections of virus, the opsonic content of the serum had arisen to 1:6,144.

IMMUNIZATION OF MONKEYS BY INTRACEREBRAL OR INTRAVENOUS INJECTIONS OF THE POLIOMYELITIS COCCUS

We wish now to record briefly the details of an experiment which indicates that a single intracerebral injection of a markedly attenuated culture of the poliomyelitic coccus, originally isolated from the spinal fluid of a patient during life, produced immunity in a monkey to repeated injections of virulent virus. Two additional monkeys immunized with a single intracerebral injection and repeated intravenous injections of attenuated cultures of poliomyelitis coccus isolated from both human and monkey sources, have been rendered immune to repeated inoculations of several fatal doses of highly virulent virus. The details of these experiments are summarized in the protocols which follow. In this connection it seems important to refer briefly to the recent work of Amoss[11] on the immunization of monkeys with cultures of the globoid bodies. Eleven monkeys received regular weekly injections of mass cultures of 4 strains of the globoid bodies over a period of 2 years. Some were injected intracerebrally, others intravenously, intraspinally and intraperitoneally. None of these monkeys developed poliomyelitis and their serum apparently did not acquire neutralizing properties against the virus of poliomyelitis. Amoss concludes that the response to antibody formation is small and at most leads to but slight reactions of agglutination and complement deviation with the cultures.

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[10] Jour. Am. Med. Assn., 1916, 67, p. 1935.

Exper. 1.—Monkey 16 (M. rhesus). April 13, 1917: Monkey etherized and given an intracerebral injection of 1 c c of a heavy suspension of mixed subcultures in the fourth generation originally isolated from the spinal fluid of patents with poliomyelitis. These strains had been cultured in the laboratory for a month and grew as fine green colonies on blood-agar plates.

May 7: Well. An intravenous injection of the surface growth of 3 agar slants of a third generation culture of the coccus from monkey Virus 312 was given.

May 21: An intravenous injection of the 24-hour surface growth of 5 agar slants of a second generation culture of the coccus isolated from monkey Virus 22 was given.

June 15: The monkey was etherized and given an intracerebral injection of 1 c c of a 5% emulsion of Virus 25.

July 19: The monkey has remained well.

July 19: Etherized and given a second intracerebral injection of 2 c c of a 5% emulsion of recently glycerolated brain and cord of monkey Virus 25 (four fatal doses).

Aug. 30: The monkey has remained well; has never shown any signs of poliomyelitis.

Exper. 2.—Monkey 35 (Ring Tail). June 27: Etherized and given an intracerebral injection of the poliomyelitis coccus isolated during life from the cerebrospinal fluid of E. H. The coccus was originally isolated in a tube of 1% dextrose broth inoculated with 1 c c of clear spinal fluid. Subcultures on standard human blood-agar plates grew as fine, slightly green colonies. The original coccus was carried through three blood-agar plates with each transplant being made from a single colony fished from the agar medium. The third generation on blood agar was inoculated into dextrose broth and subcultures were then made on agar slants. The surface growth of three agar slants was suspended in 1.5 c c of sterile salt solution and this sixth generation culture of the coccus was injected into the left cerebral hemisphere of Monkey 35.

July 19: The monkey has remained well. Was given an intracerebral injection of 1 c c of 5% emulsion of Virus 25.

Aug. 11: No symptoms to date. Etherized and given a second intracerebral injection of 1 c c of 5% emulsion of Virus 27.

Aug. 30: The monkey has never exhibited any symptoms of poliomyelitis.

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[11] Jour. Exper. Med., 1917, 25, p. 545.

Exper. 3. Monkey 18 (*M. rhesus*). April 20: Etherized and given an intracerebral injection of 1 c c of the sedimented broth culture of Virus 25 now in the fourth generation.

May 7: Has remained well. An intravenous injection of the surface growth of 3 agar slants of a third generation culture of the coccus from monkey Virus 312 was given.

May 21: Given an intravenous injection of the surface growth of 4 agar slants of a fifth generation culture of the coccus from Virus 22.

June 15: Etherized and given an intracerebral injection of 0.5 c c of a 5% emulsion of Virus 25.

Aug. 2: No symptoms. Etherized and given a second intracerebral injection of 1 c c of a 5% emulsion of Virus 25.

Aug. 30: Monkey has remained well.

Exper. 4. Monkey 30 (*M. rhesus*). June 15: Etherized and 1 c c of 5% emulsion of Virus 25 injected intracerebrally.

June 19: Tremor, irritability and convulsions.

June 20: Flaccid paralysis of both legs and weakness of the arms. Died during the night. Microscopically, typical lesions of poliomyelitis.

SUMMARY

A highly potent immune serum can be produced in the horse by repeated intravenous injections of aerobic cultures of the poliomyelitic coccus.

Such a serum contains agglutinins, complement fixing bodies and especially opsonin in high titer.

Monkeys can be immunized against several fatal doses of virulent monkey-adapted virus by repeated intravenous and intracerebral injections of the poliomyelitic coccus.

The serum of the horse immunized with strains of the poliomyelitic coccus obtained from human and monkey sources possesses protective and curative properties against the virus in experimental poliomyelitis of monkeys.